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Anti-obesity Action of *Salix matsudana* Leaves (Part 1). Anti-obesity Action by Polyphenols of *Salix matsudana* in High Fat-diet Treated Rodent Animals

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In preliminary experiments, polyphenol fractions prepared from the leaves of *Salix matsudana* reduced the elevation of the rat plasma triacylglycerol level at 3 and 4 h after oral administration of a lipid emulsion containing corn oil, at a dose of 570 mg/kg. The present study examined the anti-obesity action of polyphenol fractions of *S. matsudana* leaves by testing whether the polyphenol fractions prevented the obesity induced by feeding a high-fat diet to female mice for 9 weeks. Body weights at 2–9 weeks and the final parametrial adipose tissue weights were significantly lower in mice fed the high-fat diet with 5% polyphenols of *S. matsudana* leaves than in those fed the high-fat diet alone. The polyphenols of *S. matsudana* leaves also significantly reduced the hepatic total cholesterol content, which was elevated in mice fed the high-fat diet alone. In addition, the polyphenol fractions of *S. matsudana* leaves inhibited palmitic acid uptake into brush border membrane vesicles prepared from rat jejunum and α-amylase activity, and their fractions enhanced norepinephrine-induced lipolysis in fat cells. In conclusion, it is suggested that the inhibitory effects of the flavonoid glycoside fraction of *S. matsudana* leaves on high-fat diet-induced obesity might be due to the inhibition of carbohydrate and lipid absorption from small intestine through the inhibition of α-amylase and palmitic acid uptake into small intestinal brush border membrane or by accelerating fat mobilization through enhancing norepinephrine-induced lipolysis in fat cells. Copyright © 2003 John Wiley & Sons, Ltd.

Keywords: *Salix matsudana*; α-amylase activity; brush border membrane vesicles; high-fat diet; parametrial adipose tissue weight.

INTRODUCTION

Salix matsudana Koidz (Berberidaceae) 'Hanliu' in Chinese, is mostly distributed in northern and western China, and its leaves have been used for more than 3000 years as a traditional Chinese folk drug for treating jaundice, hepatitis, rheumatism, arthritis and eczema (Jiangsu New Medical College, 1977). Though it has recently been reported that the leaves of *S. matsudana* have anti-obesity actions, the basis for this hearsay is unclear. In preliminary experiments, it was found that polyphenol fractions of *S. matsudana* leaves appeared to reduce the elevation of plasma triacylglycerol after oral administration of a lipid emulsion consisting of corn oil, cholic acid, cholesteryloleate and saline solution. Therefore, the fat balance was measured by determination of fat excretion in the faeces of mice fed a high-fat diet or a high-fat diet plus the polyphenol fraction of *S. matsudana* leaves for 3 days, and the effects of polyphenol fraction on obesity induced by high-fat diet over the long term were examined. In addition, the anti-obesity mechanisms of polyphenol fraction of the leaves of *S. matsudana* were investigated using a lipolytic assay in rat adipocytes and an assay for inhibition of α-amylase activity *in vitro*, and inhibition of fatty acid uptake into brush border membrane vesicles prepared from rat jejunum.

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MATERIALS AND METHODS

Materials. The [1-^{14}C] palmitic acid was obtained from Du Pont NEN (England). Norepinephrine was purchased from Daiichi Pharmacy Co. (Tokyo, Japan). Collagenase (type IV) was purchased from Worthington Biochemical Co. (Freehold, NJ), and bovine serum albumin (BSA) was purchased from Wako Pure Chemical Co. (Osaka, Japan) and was extracted by the method of Chen (1967) to remove free fatty acids. The triglyceride E-test and total cholesterol E-test kits were purchased from Wako Pure Chemical Co. (Osaka, Japan). Amylase was obtained from Sigma (St Louis, MO). Sephadex LH-20 was purchased from Pharmacia Biotech Co. (Sweden). Other chemicals were of reagent grade.

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Table 1. Composition of experimental diets[a]

	High fat diet	High fat diet plus 2% polyphenol fractions (g/100 g food)	High fat diet plus 5% polyphenol fractions
Beef tallow	40	40	40
Corn starch	10	10	10
Sugar	9	9	9
Mineral mixture	4	4	4
Vitamin mixture	1	1	1
Casein	36	34	31
Polyphenol fractions of *Salix matsudana*[b]	0	2	5
kcal/100 g diet	580	580	580

[a] Laboratory pellet chow: water 8 g, carbohydrate 51.3 g, protein 24.6 g, lipid 5.6 g, fibre 3.1 g, mineral mixture 6.4 g and vitamin mixture 1 g per 100 g food; 355 kcal/100 g diet.
[b] Polyphenol fractions were calculated as 4 kcal.

Plant materials. The leaves of *Salix matsudana* were obtained from Jilin Sheng in China and voucher samples are stored at the Second Department of Medical Biochemistry, School of Medicine, Ehime University.

Animals. Male Wistar King strain rats (5 weeks old) and female ICR strain mice (3 weeks old) were purchased from Charles River Japan (Yokohama, Japan) and CLEA Japan Inc. (Osaka, Japan), respectively, and housed for 1 week in a 12 h/12 h light/dark cycle in a temperature- and humidity-controlled room. The animals were given free access to food and water. After adaptation to the lighting conditions for 1 week, the healthy animals were used in the present experiments. The Animal Studies Committee of Ehime University approved the experimental protocol.

Estimation of plasma triacylglycerol after oral administration of lipid emulsion in rats. Male Wistar King strain rats (7 weeks old, body weight 250 g) that had fasted overnight were orally administered 3 mL of lipid emulsion consisting of corn oil (6 mL), cholic acid (80 mg), cholesteryloleate (2 g) and saline solution (6 mL) with or without the polyphenol fraction of *S. matsudana* leaves (575 mg/kg body weight). Blood was taken from the tail vein 0, 1, 2, 3 and 4 h after the oral administration of the lipid emulsion with or without the polyphenol fractions of *S. matsudana* leaves and centrifuged at 5500 × *g* for 5 min using a model KH-120 M centrifuge (Kubota, Tokyo, Japan) to obtain the plasma. The triacylglycerol was determined using a triglyceride E-test Wako kit.

Estimation of fat excretion in faeces of mice fed a high-fat diet for 3 days. The mice in the experimental groups received the high-fat diet containing 2% or 5% polyphenol fraction for 3 days. The food intake of each mouse was estimated every day, and samples of faeces were obtained from each animal at regular intervals. The triacylglycerol and total cholesterol contents in faeces were measured by extraction of 150 mg sample with $CHCl_3$–MeOH (2:1, v/v, 4 mL), the extract being concentrated under a nitrogen stream. The fat content of the residue was determined using triglyceride and total cholesterol E-Test Wako kits, respectively.

Estimation of body weight, parametrial adipose tissue weight, adipose cell size, liver weight, and hepatic triacylglycerol and total cholesterol contents. Female ICR mice (3 weeks old) were maintained in a 12 h/12 h light/dark cycle in a temperature- and humidity-controlled room. The animals were fed laboratory pellet chow (CLEA Japan Inc., Osaka, Japan) and given water *ad libitum*. The healthy mice were divided into four groups (n = 14) with each group matched for body weight after 1 week of feeding. The control mice continued to be fed the laboratory pellet chow *ad libitum*. The basic composition of the experimental diet was as follows (g/100 g food): beef tallow 40, corn starch 10, sugar 9, vitamin mixture 1 and mineral mixture 4. The composition of the diet for each experimental group is shown in Table 1. Previously it was reported that reduction of casein in the high-fat diet from 36% to 31% did not affect either body weight or parametrial adipose tissue weight (Han *et al.*, 1999). Based on these facts, the flavonoid glycoside fractions of *S. matsudana* leaves was added to the high-fat diet instead of casein. To avoid auto-oxidation of their fat contents, the feeds were stored at −30 °C and freshly prepared each day. Each mouse was weighed once a week and the weight recorded. The total amount of food intake by each mouse was recorded at least three times a week. After 9 weeks of consuming the indicated experimental diet, the blood of each mouse was taken by venous puncture under anaesthetization with diethyl ether, and then the mouse was killed with an overdose of diethyl ether administered for about 2 min. The livers and parametrial adipose tissues were quickly removed and weighed, and liver tissues were stored at −80 °C until analysis. The adipose tissue shreds, each 100 mg in weight, were immediately plunged into a plastic tube containing 1.5 mL of 2% osmium tetroxide in 0.05 M collidine–HCl buffer at pH 7.4, and the tissues were fixed at 37 °C for 72 h. After fixation, the contents of the plastic container were thoroughly washed through a nylon screen (250 μm) with distilled water. The filtrate contained most of the fixed free cells, but fibrous tissue and some intact shreds of fixed adipose tissue remained on the filter. The tissue shreds were gently rubbed by hand on the filter, and the washing was continued. This procedure completely separated the tissue into free cells,

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and resulted in total recovery of cells in the filtrate. These cells were then collected and washed with distilled water by using a finer screen (25 μm). The diameter of adipose cells was determined by examining osmium tetroxide-fixed cells by scanning electron micrography (Hitachi H-500). The liver triacylglycerol contents were measured as follows: a portion (0.5 g) of the liver tissue was homogenized in Krebs Ringer phosphate buffer (pH 7.4, 4.5 mL), the homogenate (0.2 mL) was extracted with $CHCl_3$–MeOH (2:1, v/v, 4 mL), and the extract was concentrated under a nitrogen stream. The residue was analysed using Triglyceride E-test and Total Cholesterol E-test Wako kits.

Preparation of polyphenol fractions from *S. matsudana* leaves. The dry leaves (5 kg) of *S. matsudana* were extracted with petroleum ether (12 L × 2 times) for 3 h under reflux. The combined petroleum ether extracts were concentrated *in vacuo* to give a dark reddish brown extract (78 g). The residue was extracted with 95% EtOH (10 L × 3 times) for 3 h under reflux. The combined 95% EtOH extracts were concentrated *in vacuo* to give a brown extract (655 g). A portion (500 g) of the 95% EtOH extract was suspended in distilled water (2.5 L), extracted with benzene (1 L × 3 times), CH_2Cl_2 (1 L × 3 times), EtOAc (1 L × 3 times), *n*-BuOH (1 L × 3 times) and divided into benzene-, CH_2Cl_2-, EtOAc-, and *n*-BuOH-soluble and -insoluble fractions, which were concentrated *in vacuo* to give 47 g, 142 g, 33 g, 141 g, 235 g residues, respectively. The *n*-BuOH-soluble fraction (10 g) was suspended in MeOH, and chromatographed over Sephadex LH-20 (column: 1.5 × 90 cm) and developed with MeOH. The MeOH extract gave a red colour by adding Mg powder and 0.5 N HCl and a dark blue colour by adding $FeCl_3$. Therefore, these fractions were named as polyphenol fractions.

Preparation of fat cells. Young male Wistar rats were killed by cervical dislocation, and their epididymal adipose tissue was quickly removed. Fat cells were isolated from the adipose tissue by the method of Rodbell (1964).

Measurement of norepinephrine-induced lipolysis in fat cells. An aliquot of the fat cell fraction (50 μL packed volume) was incubated for 1 h at 37 °C in 200 μL of Hanks balanced solution (pH 7.4) supplemented with 2.5% BSA, norepinephrine (25 μL, final concentration: 0.05 μg/mL) and the indicated amounts of test compounds (25 μL). The release of free fatty acid (FFA) was measured as described previously (Okuda *et al.*, 1986). Briefly, the incubation mixture (250 μL) was mixed with 3 mL of chloroform/*n*-heptane (1:1, v/v) containing 2% methanol, and extracted by shaking the tube horizontally for 10 min in a shaker. The mixture was centrifuged at 2000 × *g* at 25 °C for 5 min, and the upper aqueous phase was removed by suction, and copper reagent (1 mL) was added to the lower organic phase. Then the tube was shaken for 10 min, the mixture was centrifuged at 2000 × *g* at 25 °C for 10 min, and 0.5 mL of the upper organic phase (which contained the copper salts of the extracted fatty acid) was treated with 0.5 mL of 0.1% (w/v) bathcurproine in chloroform containing 0.05% (w/v) 3-(2)-tertbutyl-4-hydroxyanisol. The absorbance of the solution was then measured at 480 nm. Lipolysis was expressed as μmole of FFA released per mL of packed fat cells per h.

Measurement of α-amylase activity *in vitro*. Soluble starch (0.4 mg/mL) in 0.25 M phosphate buffer (pH 7.0) was used as the substrate. The assay system contained the following components in a total volume of 2.02 mL: 1.0 mL substrate solution, 1.0 mL test compound solution and 0.02 mL amylase solution (final concentration 0.03 μg/mL). Incubation was carried out at pH 7.0 and 37 °C for 7.5 min. The mixture was treated with 1.0 mL of 0.01 N I_2 solution and 5.0 mL of distilled water. Its absorbance was then measured at 660 nm.

Lipid absorption by brush border membrane vesicles. Brush border membrane vesicles were prepared from the jejunum portion of the rat small intestine according to the method of Kessler *et al.* (1978). Donor vesicles composed of egg PC/palmitic acid (93:7 molar ratio) and a trace of [1-^{14}C] palmitic acid were made by sonicating 1.0 mL of the mixed lipid dispersion in 10 mM HEPES-Tris buffer, pH 7.5, containing 100 mM mannitol (buffer A) for 5 min at 4 °C. The assay mixture for investigating lipid absorption consisted of 0.24 mL of buffer A containing 29 nmol of palmitic acid (160 000 dpm) and the brush border membrane vesicles (48 μg of protein). Incubation was carried out for 30 min at 20 °C. After incubation, 0.1 mL of the incubation medium was diluted with 1 mL of ice-cold buffer A, and this solution was immediately filtered through 0.45 μm cellulose nitrate filters and washed four times with 1 mL of ice-cold buffer A. The filters were then dissolved as recommended by the supplier of ACS II, and their radioactivity was measured.

Statistical analysis. The results are expressed as mean ± standard error (SEM). Data were analysed by one-way analysis of variance (ANOVA), and then differences in mean values among groups were analysed using Fisher's protected LSD multiple comparison test and were considered significantly different at $p < 0.05$.

RESULTS AND DISCUSSION

Plasma triacylglycerol level after oral administration of lipid emulsion in rats

Figure 1 shows the time course of the plasma triacylglycerol level after oral administration of lipid emulsion. At 3 h and 4 h after oral administration of lipid emulsion, the polyphenol fractions of *S. matsudana* leaves significantly reduced the plasma triacylglycerol level. Elevation of plasma total cholesterol was caused by oral administration of lipid emulsion. The polyphenol fractions did not affect the plasma total cholesterol level after oral administration of lipid emulsion (data not shown).

Faecal triacylglycerol and total cholesterol in mice fed a high-fat diet for 3 days

The consumption of a high-fat diet plus the polyphenol fractions of *S. matsudana* leaves enhanced triacylglycerol and total cholesterol excretion in the faeces and inhibited absorption of the ingested dietary fat (Table 2).

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Table 2. Effects of the polyphenol fractions of *S. matsudana* leaves on triacylglycerol and total cholesterol excretion into faeces of mice fed a high-fat diet for 3 days

	Triacylglycerol (μmol/total faeces/day)	Total cholesterol (μmol/total faeces/day)
Normal mice	5.04 ± 0.40*	13.73 ± 0.91*
High-fat diet group	3.42 ± 0.23	4.19 ± 0.54
High-fat plus 2% polyphenol fraction group	3.17 ± 1.02	5.82 ± 0.31
High-fat plus 5% polyphenol fraction group	6.68 ± 0.49*	6.88 ± 0.47*

Results are expressed as the mean ± SEM, $n = 4$. * $p < 0.05$, significantly different from a high-fat diet group.

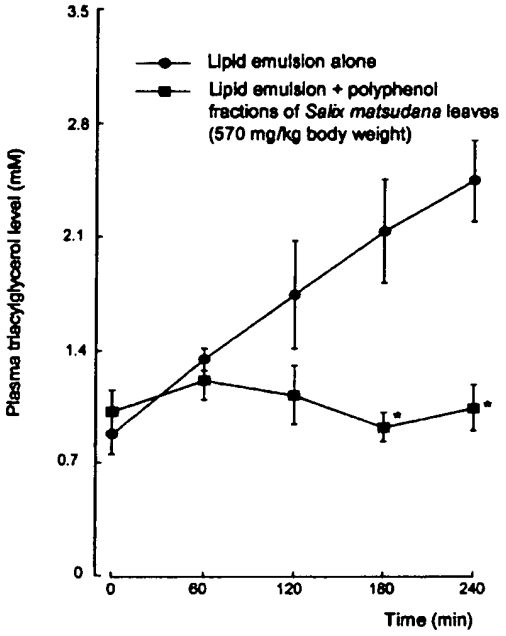

Figure 1. Effects of polyphenol fractions of *S. matsudana* leaves on rat plasma triacylglycerol level after oral administration of a lipid emulsion. Each point represents the mean ± SEM, $n = 4$. * $p < 0.05$, significantly different from a high-fat diet group.

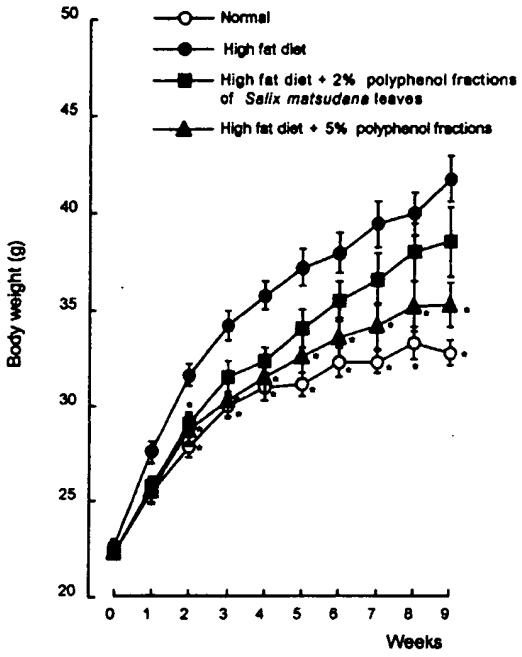

Figure 2. Effects of polyphenol fractions of *S. matsudana* leaves on body weight in mice fed a high-fat diet for 9 weeks. Results are expressed as the mean ± SEM, $n = 14$. * $p < 0.05$, significantly different from a high-fat diet group.

Body, parametrial adipose tissue and liver weights, liver triacylglycerol and total cholesterol, food consumption and adipose cell size in mice fed a high-fat diet for 9 weeks

Figure 2 shows the changes in body weight of the groups during the experiment. Consumption of a high-fat diet containing 40% beef tallow for 9 weeks produced significant increases in body weight and parametrial adipose tissue weight compared with consumption of laboratory pellet chow (control group) (Figs 2 and 3). Furthermore, the high-fat diet also induced fatty liver with an accumulation of triacylglycerol and total cholesterol compared with the control group (Table 3). Consumption of a high-fat diet containing 5% polyphenol fractions of *S. matsudana* significantly reduced the increases in body and final parametrial adipose tissue weights compared with those in the high-fat diet group. Consumption of a high-fat diet containing 2% polyphenol fractions also tended to reduce the increases in body and final parametrial adipose tissue weights, but the differences were not significant. Consumption of a high-fat diet plus polyphenol fractions significantly reduced the hepatic total cholesterol content, and also tended to reduce the hepatic triacylglycerol content, although the difference was not significant compared with the high-fat diet group (Table 3). The rate of reduction in body weigh corresponded to that of the reduction in parametrial adipose tissue weight. The mean food consumption per week per mouse during the whole experimental period was significantly different between the laboratory chow diet and high-fat diet groups, being 424.2 ± 4.6 kJ/week/mouse in the laboratory chow diet group and 578.3 ± 15.4 kJ/week/mouse in the high-fat diet group. There was no significant difference in food consumption between the high-fat diet group (578.3 ± 15.4 kJ/week/mouse) and the high-fat diet plus 2% polyphenol fractions group (570.1 ± 20.6 kJ/week/mouse) or high-fat diet plus 5%

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Table 3. Effects of the polyphenol fractions of *S. matsudana* leaves on liver weight, hepatic triacylglycerol and total cholesterol content in mice fed a high-fat diet for 9 weeks

	Liver weight (g/100 g body weight)	Triacylglycerol (μmol/g liver)	Total cholesterol (μmol/g liver)
Normal mice	4.8 ± 0.15*	18.9 ± 1.1*	7.5 ± 0.26*
High-fat diet group	6.0 ± 0.25	164.8 ± 8.9	14.5 ± 0.36
High-fat plus 2% polyphenol fraction group	5.9 ± 0.26	143.5 ± 14.0	10.6 ± 0.36*
High-fat plus 5% polyphenol fraction group	5.7 ± 0.21	138.6 ± 13.6*	10.1 ± 0.39*

Results are expressed as the mean ± SEM, $n = 14$. * $p < 0.05$, significantly different from a high-fat diet group.

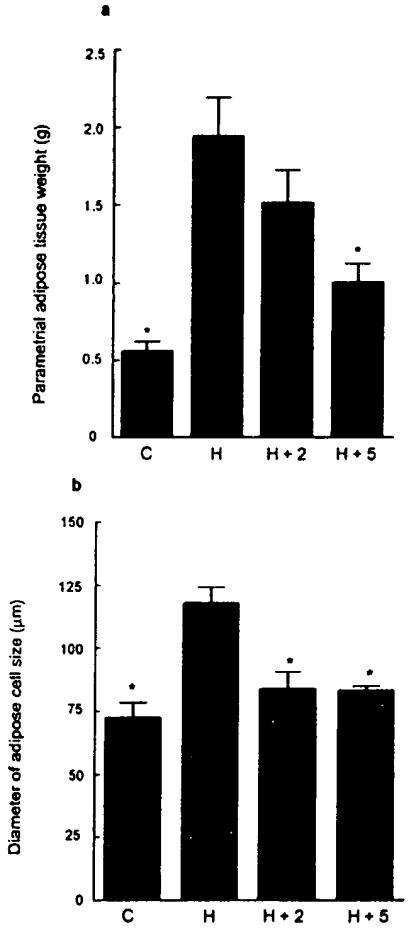

Figure 3. Effects of polyphenol fractions of *S. matsudana* leaves on parametrial adipose tissue weight (a) and diameter of adipose cells (b) in mice fed a high-fat diet for 9 weeks. C, control group; H, high-fat diet; H + 2, high-fat diet plus 2% polyphenol fractions of *S. matsudana* leaves; H + 5, high-fat diet plus 5% polyphenol fractions. Results are expressed as the mean ± SEM, $n = 14$. * $p < 0.05$, significantly different from a high-fat diet group.

polyphenol fractions group (571.0 ± 19.4 kJ/week/mouse). The diameter of adipose cells was significantly greater in the high-fat diet group than that in the control group, and the diameter in the high-fat diet plus polyphenol fraction-treated group was increased less than that in the group fed the high-fat diet only (Fig. 3).

Table 4. Effects of 95% EtOH and polyphenol fraction of *S. matsudana* leaves on norepinehrine-induced lipolysis in isolated fat cells

Addition (/mL reaction mixture)	% of control
None	0 ± 0*
Norepinephrine (Norepi) (0.05 μg)	100.0 ± 1.5
Norepi + 95% EtOH extract (1 mg)	131.9 ± 2.4*
Norepi + Non-polyphenol fraction (1 mg)	95.3 ± 5.0
Norepi + Polyphenol fraction (1 mg)	126.1 ± 2.5*

Results are expressed as the mean ± SEM, $n = 4$. * $p < 0.05$, significantly different from norepinephrine alone.

Norepinephrine-induced lipolysis

The 95% EtOH extract of *S. matsudana* leaves enhanced norepinephrine-induced lipolysis at a concentration of 1 mg/mL (Table 4), while it did not cause lipolysis in the absence of norepinephrine (data not shown). The *n*-BuOH-soluble fraction separated from the 95% EtOH extract and polyphenol fractions prepared from *n*-BuOH-soluble fraction also enhanced norepinephrine-induced lipolysis at a concentration of 1 mg/mL, but non-polyphenol fractions had no effect on norepinephrine-induced lipolysis.

α-Amylase activity by flavonoid glycoside fractions

It has been reported that a α-amylase inhibitor from wheat flour prevented obesity through inhibition of digestion and absorption of carbohydrates (Yokota *et al.*, 1994; Lankisch *et al.*, 1998). It was found that the polyphenol fractions inhibited amylase activity at concentrations of 250–5000 μg/mL (Table 5).

Table 5. Effects of polyphenol fractions of *S. matsudana* leaves on α-amylase activity

Addition (/mL reaction mixture)	α-Amylase activity (% of control)
None	100.0 ± 2.5
Polyphenol fractions (250 μg)	98.2 ± 0.8
(2500 μg)	61.3 ± 1.1*
(5000 μg)	20.0 ± 0.5*

Results are expressed as the mean ± SEM, $n = 4$–8. * $p < 0.05$, significantly different from no addition (none).

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Table 6. Effects of polyphenol fractions of *S. matsudana* leaves on palmitic acid uptake into brush border membrane vesicles of rat jejunum

Addition (/mL reaction mixture)	Palmitic acid uptake to small intestinal brush border membrane (% of control)
None	100.0
Polyphenol fractions (250 μg)	81.4
(500 μg)	0.0
(1000 μg)	0.0

Results are expressed as the mean, $n = 2$.

Palmitic acid uptake into brush border membrane vesicles of rat small intestine *in vitro*

The polyphenol fractions completely inhibited the incorporation of palmitic acid into brush border membrane vesicles at concentrations of 500 and 1000 μg/mL (Table 6).

There are a number of studies describing high-fat diet-induced obesity (Flatt, 1987; Awad *et al.*, 1990; Shimomura *et al.*, 1990; Hill *et al.*, 1993). Though it has recently been reported that the leaves of *S. matsudana* have anti-obesity actions, the basis for this hearsay is unclear. Therefore, experiments were designed to clarify whether high-fat diet-induced obesity in female mice may be prevented by *S. matsudana* leaves, possibly due to inhibition of the intestinal absorption of dietary fat and carbohydrates. In the present study, it was found that polyphenol fractions of *S. matsudana* leaves prevented the increases in body and parametrial adipose tissue weights in mice fed a high-fat diet containing 40% beef tallow for 9 weeks. The high-fat diet caused the accumulation of liver triacylglycerol and total cholesterol compared with the control group. The hepatic total cholesterol content was reduced by the administration of the polyphenol fractions compared with the high-fat diet-treated group, and the hepatic triacylglycerol content was also reduced but not significantly. On the other hand, the liver weight was nearly the same between the high-fat diet and high-fat diet plus polyphenol fraction-treated groups. The mean food consumption per week per mouse during the whole experimental period was significantly different between the laboratory chow and high-fat diet groups, but not significantly different between the high-fat diet and high-fat diet plus polyphenol fraction-treated groups. These results suggest that the polyphenol fractions of *S. matsudana* leaves might exert their anti-obesity action through inhibition of intestinal absorption of dietary fat and carbohydrates, acceleration of lipolysis in adipose tissue, and by other mechanisms. It is well known that dietary fat is not absorbed from the intestine unless it has been subjected to the action of pancreatic lipase (Verger, 1984). Polyphenol fractions of *S. matsudana* leaves did not affect pancreatic lipase activity *in vitro* (data not shown), whereas they inhibited the absorption of palmitic acid by small intestinal brush border membrane vesicles. These results suggest that the polyphenol fractions of *S. matsudana* leaves may reduce the intestinal absorption of dietary fat by inhibiting the absorption of palmitic acid, one of the products of hydrolysis of dietary fat. In fact, the polyphenol fractions were confirmed to significantly reduce the plasma triacylglycerol level that was elevated due to oral administration of a lipid emulsion containing corn oil. These results suggest that the reduction of plasma triacylglycerol levels by the polyphenol fractions of *S. matsudana* leaves may be mediated through inhibition of the intestinal absorption of palmitic acid produced by the hydrolysis of corn oil. The accumulation of fat in the liver and in parametrial adipose tissue induced by a high-fat diet was reduced by the consumption of the polyphenol fractions, perhaps through inhibition of the intestinal absorption of carbohydrate by the inhibition of α-amylase, or through stimulation of excretion of ingested dietary fat in the faeces, or the stimulation by polyphenol fractions of norepinephrine-induced lipolysis in adipocytes. These results conclude that the inhibitory effects of polyphenol fraction of *S. matsudana* leaves on high-fat diet-induced obesity might be due to the inhibition of carbohydrate and lipid absorption from the small intestine through the inhibition of α-amylase and palmitic acid uptake into small intestinal brush border membrane or accelerating fat mobilization through enhancing norepinephrine-induced lipolysis in fat cells.

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